Am ndment to the Title

Please cancel the present title and replace it with the following new title:

Integrated Nucleic Acid Diagnostic Device and Method ~~Designed~~ For In-Situ Confocal Microscopy

Amendments to the Specification

On page 15, please replace the first complete paragraph with the following:

While oligonucleotide probes may be prepared having every possible sequence of length n, it will often be desirable in practicing the present invention to provide an oligonucleotide array which is specific and complementary to a particular nucleic acid sequence. For example, in particularly preferred aspects, the oligonucleotide array will contain oligonucleotide probes which are complementary to specific target sequences, and individual or multiple mutations of these. Such arrays are particularly useful in the diagnosis of specific disorders which are characterized by the presence of a particular nucleic acid sequence. For example, the target sequence may be that of a particular exogenous disease causing agent, e.g., human immunodeficiency virus (see, U.S. application Ser. No. 08/284,064, now abandoned, previously incorporated herein by reference), or alternatively, the target sequence may be that portion of the human genome which is known to be mutated in instances of a particular disorder, i.e., sickle cell anemia (see, e.g., U.S. application Ser. No. 08/082,937, now abandoned, previously incorporated herein by reference) or cystic fibrosis.

On page 18, please replace the paragraph starting on line 19 with the following:

Following the data gathering operation, the data will typically be reported to a data analysis operation. To facilitate the sample analysis operation, the data obtained by the reader from the device will typically be analyzed using a digital computer. Typically, the computer will be

appropriately programmed for receipt and storage of the data from the device, as well as for analysis and reporting of the data gathered, i.e., interpreting fluorescence data to determine the sequence of hybridizing probes, normalization of background and single base mismatch hybridizations, ordering of sequence data in SBH applications, and the like, as described in, e.g., U.S. patent application Ser. No. 08/327,525, filed Oct. 21, 1994, issued as US Patent 5,795,716, and incorporated herein by reference.

On page 20, please replace the first paragraph with the following:

As a miniaturized device, the body of the device will typically be approximately 1 to 10 cm in length by about 1 to 10 cm in width by about 0.2 to about 2 cm thick. Although indicative of a rectangular shape, it will be readily appreciated that the devices of the invention may be embodied in any number of shapes depending upon the particular need. Additionally, these dimensions will typically vary depending upon the number of operations to be performed by the device, the complexity of these operations and the like. As a result, these dimensions are provided as a general indication of the size of the device. The number and size of the reaction chambers included within the device will also vary depending upon the specific application for which the device is to be used. Generally, the device will include at least two distinct reaction chambers, and preferably, at least three, four or five distinct reaction chambers, all integrated within a single body. Individual reaction chambers will also vary in size according to the specific function of the reaction chamber. In general however, the reaction chambers will be from about 0.5 to about 20 mm in width or diameter and about 0.05 to about 5 mm deep. Fluid channels, on the other hand, typically range from about 20 to about 1000 μm wide, preferably, 100 to 500 μm wide and about 5 to 100 μm deep.

On page 21, please replace the paragraph starting on line 3 with the following:

Photolithographic methods of etching substrates are particularly well suited for the microfabrication of these ~~hese~~ substrates and are well known in the art. For example, the first

sheet of a substrate may be overlaid with a photoresist. An electromagnetic radiation source may then be shone through a photolithographic mask to expose the photoresist in a pattern which reflects the pattern of chambers and/or channels on the surface of the sheet. After removing the exposed photoresist, the exposed substrate may be etched to produce the desired wells and channels. Generally preferred photoresists include those used extensively in the semiconductor industry. Such materials include polymethyl methacrylate (PMMA) and its derivatives, and electron beam resists such as poly(olefin sulfones) and the like (more fully discussed in, e.g., Ghandi, "VLSI Fabrication Principles," Wiley (1983) Chapter 10, incorporated herein by reference in its entirety for all purposes).

On page 22, please replace the paragraph starting on line 11 with the following:

In particularly preferred embodiments, the body of the device is made from at least one injection molded, press molded or machined polymeric part that has one or more wells or depressions manufactured into its surface to define several of the walls of the reaction chamber or chambers. Examples of suitable polymers for injection molding or machining include, e.g., polycarbonate, polystyrene, polypropylene, polyethylene acrylic, and commercial polymers such as Kapton, Valox, Teflon, ABS, Delrin and the like. A second part that is similarly planar in shape is mated to the surface of the polymeric part to define the remaining wall of the reaction chamber(s). U.S. patent application Ser. No. 08/528,173, filed Sep. 15, 1995, issued as US Patent 6,140,044, incorporated herein by reference, describes a device that is used to package individual oligonucleotide arrays. The device includes a hybridization chamber disposed within a planar body. The chamber is fluidly connected to an inlet port and an outlet port via flow channels in the body of the device. The body includes a plurality of injection molded planar parts that are mated to form the body of the device, and which define the flow channels and hybridization chamber.

On page 35, please replace the paragraph starting on line 19 with the following:

Temperature controlled reaction chambers will also typically include a miniature temperature sensor for monitoring the temperature of the chamber, and thereby controlling the application of current across the heater. A wide variety of microsensors are available for determining temperatures, including, e.g., thermocouples having a bimetallic junction which produces a temperature dependent electromotive force (EMF), resistance thermometers which include material having an electrical resistance proportional to the temperature of the material, thermistors, IC temperature sensors, quartz thermometers and the like. See, Horowitz and Hill, The Art of Electronics, Cambridge University Press 1994 (2nd Ed. 1994). One heater/sensor design that is particularly suited to the device of the present invention is described in, e.g., U.S. patent application Ser. No. 08/535,875, filed Sep. 28, 1995, issued as US Patent 6,132,580, and incorporated herein by reference in its entirety for all purposes. Control of reaction parameters within the reaction chamber, e.g., temperature, may be carried out manually, but is preferably controlled via an appropriately programmed computer. In particular, the temperature measured by the temperature sensor and the input for the power source will typically be interfaced with a computer which is programmed to receive and record this data, i.e., via an analog-digital/digital-analog (AD/DA) converter. The same computer will typically include programming for instructing the delivery of appropriate current for raising and lowering the temperature of the reaction chamber. For example, the computer may be programmed to take the reaction chamber through any number of predetermined time/temperature profiles, e.g., thermal cycling for PCR, and the like. Given the size of the devices of the invention, cooling of the reaction chambers will typically occur through exposure to ambient temperature, however additional cooling elements may be included if desired, e.g., coolant systems, peltier coolers, water baths, etc.

On page 46, please replace the first paragraph with the following:

The efficacy of acoustic mixing was also tested in an actual hybridization protocol. For this hybridization test, a fluorescently labeled oligonucleotide target sequence having the sequence 5'-GAGATGCGTCGGTGGCTG-3' and an array having a checkerboard pattern of 400 μm squares having complements to this sequence synthesized thereon, were used. Hybridization of a 10 nM solution of the target in 6xSSPE was carried out. During hybridization, the external surface of the array was kept in contact with a thermoelectric cooler set at 15 °C. Hybridization

was carried out for 20 minutes while driving the crystal at 2 MHz at an average power of 4 W (on time=0.2 sec., off time=0.8 sec.). The resulting average intensity was identical to that achieved using mechanical mixing of the chamber (vertical rotation with an incorporated bubble).